FINAL REPORT

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PRINCIPAL INVESTIGATOR: Michael R. Silverman, Ph.D.

INSTITUTION: The Agouron Institute
505 Coast Blvd. South
La Jolla, CA 92037

GRANT TITLE: Sensory Mechanisms Controlling Bacterial Bioluminescence

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OBJECTIVE: The goal of this research was to explore the sensory mechanisms which control expression of bioluminescence in the marine bacterium *Vibrio harveyi*. Examination of sensory control focused on the genetic regulatory pathways which respond to extracellular signals such as autoinducers and intracellular signals such as nutrient and trace element availability, oxygen tension and redox or other general indicators of the metabolic state of the cell.

APPROACH: Regulatory genes were isolated by reconstructing a system which expresses luminescence in a recombinant *E. coli* host. This required positioning the cloned *luxCDABEGH* operon encoding the luminescence enzymes and the *luxR* gene encoding an obligatory transcription factor in *E. coli* and then adding a recombinant library containing fragments of the *V. harveyi* genome. Particular cloned genes which stimulate expression of the lux operon were identified and subjected to further analysis, and transposon and chemical mutagenesis was applied to *V. harveyi* to isolate mutants with interesting regulatory phenotypes. The mutants were employed to isolate wild type, functional copies of the regulatory genes. The cloned genes obtained by both approaches were sequenced and mutated to generate defined, site-specific, missense and null defects, and the mutated genes were transferred into the genome of *V. harveyi* for extensive phenotype analysis.

ACCOMPLISHMENTS: We identified three different genetic loci which stimulated light production by the reconstituted luminescence system in recombinant *E. coli* containing *luxR* and the *luxCDABEGH* operon. These loci, named *luxS*, *luxT* and *luxU*, contain candidates for genes which encode functions important for sensory control. We initially focused on *luxT* which has been shown to activate transcription of *luxR*. It was mapped and sequenced. Analysis of the DNA sequence and the derived protein sequence revealed that *luxT* has a high degree of similarity (65%

identity at the DNA level and 71% identity at the protein level) to the *E. coli* regulatory gene *gcvA*. *GcvA* is a member of the *lysR* gene family and functions in *E. coli* to regulate the expression of genes involved in the catabolism of glycine. The implication of the similarity of *luxT* and *gcvA* is that luminescence is subject to metabolic control by the concentration of amino acids (and purines) in its environment. We constructed a mutant of *V. harveyi* using a gene replacement method, but the mutant is not defective in the expression of luminescence under the nutritional conditions examined so far. More work will be needed to determine what role *luxT* has in controlling luminescence. We also made many attempts to use a recombinant library to complement the defect in a mutant which does not produce an extracellular autoinducer substance (specifically AI-2). Such a complementing clone should contain the genes encoding the synthesis of this as-yet unidentified substance. However, cloning attempts were not successful and further work is required to exploit the signalling mutant.

CONCLUSIONS: Genetic studies of the regulation of bacterial bioluminescence have been very rewarding. The genetic mechanism controlling density-dependent expression of luminescence, i.e. "quorum sensing", has become a paradigm for understanding the control of diverse functions in many genera of bacteria. However, other aspects of regulation such as control of luminescence by nutritional or metabolic signals are mysterious. Identification and characterization of the signalling systems which respond to such signals could lead to a better understanding of the function of luminescence, and analysis of lux regulation could also reveal novel and fundamentally important control mechanisms. How does a cell perceive its state of well-being and decide to glow, or grow or move? Much remains to be learned about the sensory mechanisms in bacteria.

SIGNIFICANCE: This work helps us understand how marine bacteria adapt to their environment by sensing the conditions which surround them. Mechanism discovered with these bacteria should help us understand other organisms as well.

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Michael R. Silverman, Ph.D.

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Agouron Institute
505 Coast Boulevard South
La Jolla, CA 92037

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The goal of this project was to explore the sensory mechanisms which control the expression of bioluminescence in the marine bacterium *Vibrio harveyi*. Genetic methods were used to discover the genes which encode functions for the production of extracellular, chemical signals (autoinducers) and for the synthesis of proteins which mediate the response to such signals. A mutant defective in the production of one class of autoinducers was isolated. The gene or genes defective in this mutant were not isolated, but this mutant should be useful for future work to identify specific signaling functions. Another gene, *luxT*, which is important for transcription of the *lux* genes encoding the luminescence enzymes, was cloned and sequenced. Further work to understand its precise role must still be done.

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